


I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on March 27, 2009.  
  
Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF  
CORRECTION UNDER 37 CFR 1.322  
AND UNDER 37 CFR 1.323  
Docket No. G.194US03PCT  
Patent No. 7,442,519

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Laurent Cavarec, Ilya Chumakov, Benoit Destenaves, Catherine Gonthier,  
Isabelle Elias  
Issued : October 28, 2008  
Patent No. : 7,442,519  
Conf. No. : 9318  
For : KCNQ2-15 Potassium Channel

Mail Stop Certificate of Corrections Branch  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION  
UNDER 37 CFR 1.322 (OFFICE MISTAKE)  
UNDER 37 CFR 1.323 (APPLICANT MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

**Patent Reads:**

Column 12, line 62:

“ “Phenotype” ”

**Application Reads:**

Page 17, line 3:

--“phenotype”--

**Patent Reads:**Column 14, line 2:

“such assay s”

Column 14, line 12:

“alter native”

**Patent Reads:**Column 16, line 2:

“ncbi.nlm.nih.gov)”

**Patent Reads:**Column 17, line 47:

“substancially the same”

Column 18, line 37:

“complementary thereto”

Column 18, line 43:

“complementary thereto”

Column 18, lines 49-50:

“to a polynucleotides”

**Patent Reads:**Column 18, line 63:

“NO; 3”

Column 23, line 23:

“Accession No. 043526”

**Application Should Read:**Page 18, line 20:

--such assays--

Page 18, line 26:

--alternative--

**Application Reads:**Page 21, line 1:

--ncbi.nlm.nih.gov)--

**Application Should Read:**Page 22, line 51:

--substantially the same--

Page 24, line 3:

--complementary thereto--

Page 24, line 6:

--complementary thereto--

Page 24, line 11:

--to a polynucleotide--

**Application Reads:**Page 24, line 20:

--NO: 3--

Page 30, line 2:

--Accession No. O43526--

**Patent Reads:**Column 25, lines 11-12:

“ho momeric”

**Patent Reads:**Column 25, line 53:

“that (I)”

Column 28, line 56:

“consisting of 30-2162”

Column 30, line 1:

“marker 30-7130”

Column 30, line 67:

“Human foetal”

Column 31, line 33:

“SDI-Leu/-Trp and SDI-Leu/-Trp/-His/-Ade”

**Patent Reads:**Column 31, line 35:

“the He Yeast”

**Patent Reads:**Column 33, line 32:

“digestion With EcoRi”

Column 33, line 61:

“pGAD7”

**Application Should Read:**Page 32, line 12:

--homomeric--

**Application Reads:**Page 33, line 3:

--that (i)--

Page 37, line 1:

--consisting of 30-2/62--

Page 38, line 20:

--marker 30-7/30--

Page 40, line 5:

--Human foetal--

Page 40, line 30:

--SD/-Leu/-Trp and SD/-Leu/-Trp/-His/-Ade--

**Application Should Read:**Page 40, line 32:

--the Yeast--

**Application Reads:**Page 42, line 37:

--digestion with EcoRi--

Page 43, line 19:

--pGADT7--

Column 34, line 42:

“10  $\mu$ l”

Column 34, line 44:

“SDI-Leu/-Trp/-His/-Ade”

Column 36, line 50:

“w performed”

**Patent Reads:**

Column 37, lines 19-20:

“membrane were then blocked”

Column 37, line 66:

“phosphorylation”

Column 39, line 9:

“phosphorylation”

**Patent Reads:**

Column 40, line 17:

“1  $\mu$ d”

Column 45, line 57:

“10  $\mu$ mol”

Column 46, line 20:

“dassification”

Column 50, line 64:

“for 30-7130”

Page 44, line 4:

--100  $\mu$ l--

Page 44, line 5:

--SD/-Leu/-Trp/-His/-Ade--

Page 46, lines 27-28:

--was performed--

**Application Should Read:**

Page 47, line 13:

--membrane was then blocked--

Page 48, line 7:

--phosphorylation--

Page 49, line 24:

--phosphorylation--

**Application Reads:**

Page 51, line 3:

--1  $\mu$ l--

Page 57, line 28:

--10 pmol--

Page 58, line 8:

--classification--

Page 63, line 13:

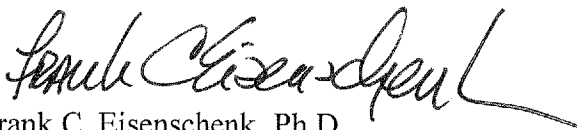
--for 30-7/30--.

A true and correct copy of pages 17, 21, 24, 30, 33, 37, 38, 40, 42-44, 46, 51, 57, 58, and 63 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

The fee of \$100.00 was paid at the time this Request was filed. The Commissioner is also authorized to charge any additional fees as required under 37 CFR 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.

Patent Attorney

Registration No. 45,332

Phone No.: 352-375-8100

Fax No.: 352-372-5800

Address: P.O. Box 142950

Gainesville, FL 32614-2950

FCE/jb

Attachments: Copy of pages 17, 21, 24, 30, 33, 37, 38, 40, 42-44, 46, 51, 57, 58, and 63 of the specification

terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

The terms "trait" and "phenotype" are used interchangeably herein and refer to any clinically distinguishable, detectable or otherwise measurable property of an organism such as symptoms of, or susceptibility to a disease for example. Typically the terms "trait" or "phenotype" are used herein to refer to symptoms of, or susceptibility to bipolar disorder; or to refer to an individual's response to an agent acting on bipolar disorder; or to refer to symptoms of, or susceptibility to side effects to an agent acting on bipolar disorder.

As used herein, the term "allele" refers to one of the variant forms of a biallelic marker, differing from other forms in its nucleotide sequence. Typically the first identified allele is designated as the original allele whereas other alleles are designated as alternative alleles. Diploid organisms may be homozygous or heterozygous for an allelic form.

The term "polymorphism" as used herein refers to the occurrence of two or more alternative genomic sequences or alleles between or among different genomes or individuals. "Polymorphic" refers to the condition in which two or more variants of a specific genomic sequence can be found in a population. A "polymorphic site" is the locus at which the variation occurs. A polymorphism may comprise a substitution, deletion or insertion of one or more nucleotides. A single nucleotide polymorphism is a single base pair change. Typically a single nucleotide polymorphism is the replacement of one nucleotide by another nucleotide at the polymorphic site. A "single nucleotide polymorphism" (SNP) refers to a sequence polymorphism differing in a single base pair.

## **2. KCNQ2-15b polypeptides of the present invention**

The term "KCNQ2-15b polypeptides" is used herein to embrace all of the polypeptides of the present invention.

Preferably, the KCNQ2-15b is selected from a peptide, a polypeptide or a protein selected from the group consisting of:

- a) a polypeptide comprising a span of at least ten amino acids of amino acids 589 to 643 of SEQ ID NO: 2;
- b) a polypeptide comprising amino acids 589 to 643 of SEQ ID NO: 2;
- c) a polypeptide comprising amino acids 545 to 643 of SEQ ID NO: 2;
- d) a polypeptide comprising SEQ ID NO: 2;
- e) a polypeptide comprising SEQ ID NO: 4;
- f) a polypeptide comprising SEQ ID NO: 6;

NCBI at world wide web site [ncbi.nlm.nih.gov](http://ncbi.nlm.nih.gov)) and FASTA (Pearson W R, 1990; Pearson 1988).

Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz polypeptides, may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule (Grantham, 1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, e.g. under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e.g. cysteine residues. Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined in Table III.

**TABLE I**

**Preferred Groups of Synonymous Amino Acids**

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

**TABLE II**

**More Preferred Groups of Synonymous Amino Acids**

Amino Acid	Synonymous Group
Ser	Ser

least 70, 80, 85, 90, 95, 96, 97, 98 or 99% nucleotide identity with a polynucleotide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 5, sequences complementary thereto and fragments thereof.

Another object of the invention relates to purified polynucleotides that hybridize under moderately stringent conditions or under highly stringent conditions with a polynucleotide selected from the group consisting of sequences complementary thereto and fragments thereof.

Most preferred KCNQ2-15b polynucleotides of the invention include polynucleotides encoding a KCNQ2-15bx polypeptide, a KCNQ2-15by polypeptide or a KCNQ2-15bz polypeptide. A KCNQ2-15bx polynucleotide corresponds to a polynucleotide encoding a KCNQ2-15bx polypeptide. A KCNQ2-15by polynucleotide corresponds to a polynucleotides encoding a KCNQ2-15by polypeptide. A KCNQ2-15bz polynucleotide corresponds to a polynucleotide encoding a KCNQ2-15bz polypeptide.

In some embodiments, said KCNQ2-15b polynucleotide comprises or consists of the coding sequence (CDS) encoding the KCNQ2-15b polypeptide. In other embodiments, said KCNQ2-15b polynucleotide comprises or consists of the messenger RNA (mRNA) encoding the KCNQ2-15b polypeptide. In further embodiments, said KCNQ2-15b polynucleotide comprises or consists of the complementary DNA (cDNA) encoding the KCNQ2-15b polypeptide. Preferred KCNQ2-15b polynucleotides are polynucleotides comprising a CDS having the sequence of SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5, mRNAs comprising these CDSs and cDNAs comprising these CDSs.

The present invention also encompasses fragments of KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz polynucleotides for use as primers and probes. Such primers are useful in order to detect the presence of at least a copy of a KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz polynucleotide, complement, or variant thereof in a test sample. The probes of the present invention are useful for a number of purposes. They can notably be used in Southern hybridization to genomic DNA. The probes can also be used to detect PCR amplification products. They may also be used to detect mismatches in the KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz mRNAs using other techniques. They may further be used for *in situ* hybridization.

Any of the polynucleotides, primers and probes of the present invention can be conveniently immobilized on a solid substrate, such as, e.g., a microarray. A substrate comprising a plurality of oligonucleotide primers or probes of the invention may be used either for detecting or amplifying targeted sequences in the KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz gene, may be used for detecting mutations in the coding or in the non-coding sequences of the KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz mRNAs, and may also be used to determine expression of KCNQ2-15bx, KCNQ2-15by or KCNQ2-15bz mRNAs in

KCNQ2-15b polypeptides and all previously described isoforms (see, e.g., SwissProt Accession No. O43526). As further used herein, the term "KCNQ2-fl" refers to a polypeptide of SEQ ID NO: 7.

As used herein, the term "modulator" refers to a compound that increases or decreases any of the properties of a KCNQ2 polypeptide. As used herein, a "KCNQ2 modulator" refers to a compound that increases or decreases the activity of a KCNQ2 polypeptide and/or to a compound that increases or decreases the transcription level of the KCNQ2 mRNA encoding said polypeptide. The term "modulator" encompasses both agonists and antagonists.

As used herein, a "KCNQ2 antagonist" refers to a compound that decreases the activity of a KCNQ2 polypeptide and/or to a compound that decreases the expression level of the KCNQ2 mRNA encoding said polypeptide. The terms "antagonist" and "inhibitor" are considered to be synonymous and can be used interchangeably throughout the disclosure.

As used herein, a "KCNQ2 agonist" refers to a compound that increases the activity of a KCNQ2 polypeptide and/or to a compound that increases the expression level of the KCNQ2 mRNA encoding said polypeptide. The terms "agonist" and "activator" are considered to be synonymous and can be used interchangeably throughout the disclosure.

Methods that can be used for testing modulators for their ability to increase or decrease the activity of a KCNQ2 polypeptide or to increase or decrease the expression of a KCNQ2 mRNA are well known in the art and further detailed below. Preferred modulators of the present invention are modulators of KCNQ2-15bx, KCNQ2-15by, KCNQ2-15bz or KCNQ2-fl. The assays described herein and known in the art for measuring KCNQ2 activity can be performed either *in vitro* or *in vivo*.

Candidate compounds according to the present invention include naturally occurring and synthetic compounds. Such compounds include, e.g., natural ligands, small molecules, antisense mRNAs, antibodies, aptamers and short interfering RNAs. As used herein, the term "natural ligand" refers to any signaling molecule that binds to a phosphatase comprising PP2A/By *in vivo* and includes molecules such as, e.g., lipids, nucleotides, polynucleotides, amino acids, peptides, polypeptides, proteins, carbohydrates and inorganic molecules. As used herein, the term "small molecule" refers to an organic compound. As used herein, the term "antibody" refers to a protein produced by cells of the immune system or to a fragment thereof that binds to an antigen. As used herein, the term "antisense mRNA" refers to an RNA molecule complementary to the strand normally processed into mRNA and translated, or complementary to a region thereof. As used herein, the term "aptamer" refers to an artificial nucleic acid ligand (see, e.g., Ellington and Szostak (1990) *Nature* 346:818-822). As used herein, the term "short interfering RNA" refers to a double-stranded RNA inducing sequence-

In another embodiment, the activity of a KCNQ2 polypeptide is measured by determining the phosphorylation state of the KCNQ2 polypeptide as described in example 7. In the frame of the present invention, it has been found that (i) KCNQ2 -15b polypeptides are dephosphorylated by a PP2A phosphatase comprising a PP2A/B $\gamma$  subunit, the gene encoding the PP2A/B $\gamma$  subunit being associated with bipolar disorder; and (ii) phosphorylated by GSK3 $\beta$ , a kinase that is inhibited by mood stabilizing agents. Thus the phosphorylation state of a KCNQ2 polypeptide is believed to be correlated with the biological activity of the KCNQ2 polypeptide. The phosphorylation state of a KCNQ2 polypeptide may for example be measured in an assay as described in example 7.

One preferred embodiment is directed to the use of a KCNQ2-15b polypeptide as a target for screening candidate modulators. Another preferred embodiment is directed to the use of a KCNQ2-fl polypeptide as a target for screening candidate modulators.

Modulators of KCNQ2 polypeptides, which may be found, e.g., by any of the above screenings, are candidate drugs for the treatment of a mental disorder. Thus a preferred embodiment of the present invention is the use of a KCNQ2 polypeptide as a target for screening candidate compounds for candidate drugs for the treatment of a mental disorder.

As used herein, the term "Mental disorder" includes bipolar disorder, schizophrenia, depression as well as other mood disorders and psychotic disorders. Preferably, said mental disorder is bipolar disorder, schizophrenia or depression. Most preferably, said mental disorder is bipolar disorder.

A further aspect of the present invention is the use of a modulator of a KCNQ2 polypeptide for screening for drugs for the treatment of a mental disorder. One example of a method that can be used for screening for drugs for the treatment of a mental disorder and/or for assessing the efficiency of an modulator of a KCNQ2 polypeptide for the treatment of a mental disorder is a method comprising the step of administering said modulator to an animal model for said mental disorder, wherein a determination that said modulator ameliorates a representative characteristic of said mental disorder in said animal model indicates that said modulator is a drug for the treatment of said mental disorder.

Animal models for mental disorders and assays for determining whether a compound ameliorates a representative characteristic of said mental disorder in said animal model are described and used. For example, animal models that may be used in the above method include but are not limited to the conditioned avoidance behaviour model in rats, which is a standard behavioural test predictive of antipsychotic activity, the behavioral activity assessment of mice and rats in the Omnitech Digiscan animal activity monitors, the purpose of which is to evaluate compounds for antipsychotic-like CNS effects and a variety of other behavioral effects generally associated with CNS activity, the blockade of amphetamine-stimulated locomotion in rat, the protocol for the prepulse inhibition of acoustic startle model

KCNQ2-related biallelic marker may be selected from the group consisting of 30-2/62 and 30-7/30 and the complements thereof. Alternatively, The KCNQ2-related biallelic marker may be selected from the group consisting of 30-4/58, 30-17/37, 30-84/37 and 30-15/54 and the complements thereof. The KCNQ2-related biallelic marker may also be a marker that is not specifically disclosed by the present specification. Preferably, the mental disorder is selected from the group consisting of bipolar disorder, schizophrenia and depression. Most preferably, the mental disorder is bipolar disorder.

The present invention is further directed to a method of genotyping comprising the step of determining the identity of a nucleotide at a KCNQ2-related biallelic marker or the complement thereof in a biological sample. Preferably, said biological sample is derived from a single subject. It is preferred that the identity of the nucleotides at said biallelic marker is determined for both copies of said biallelic marker present in said individual's genome. In a preferred embodiment, the identity of the nucleotide at said biallelic marker is determined by a microsequencing assay. Preferably, a portion of a sequence comprising the biallelic marker is amplified prior to the determination of the identity of the nucleotide. The amplification may preferably be performed by PCR. Such a method of genotyping may for example be performed using any of the protocols described in examples 10 to 14 of the present specification. Further methods of genotyping are well known by those of skill in the art and any other known protocol may be used.

Methods well-known to those skilled in the art that may be used for genotyping in order to detect biallelic polymorphisms include methods such as, conventional dot blot analyzes, single strand conformational polymorphism analysis (SSCP) (Orita et al. (1989) Proc Natl Acad Sci USA 86:2766-2770), denaturing gradient gel electrophoresis (DGGE) (Borresen et al. (1988) Mutat Res. 202:77-83.), heteroduplex analysis (Lessa et al. (1993) Mol Ecol. 2:119-129), mismatch cleavage detection (Grompe et al. (1989) Proc Natl Acad Sci USA. 86:5888-5892). Another method for determining the identity of the nucleotide present at a particular polymorphic site employs a specialized exonuclease-resistant nucleotide derivative as described in US patent No. 4,656,127. Oligonucleotide microarrays or solid-phase capturable dideoxynucleotides and mass spectrometry may also be used (Wen et al. (2003) World J Gastroenterol. 9:1342-1346; Kim et al. (2003) Anal Biochem. 316:251-258). Preferred methods involve directly determining the identity of the nucleotide present at a biallelic marker site by sequencing assay, microsequencing assay, enzyme-based mismatch detection assay, or hybridization assay.

As used herein, the term "biological sample" refers to a sample comprising nucleic acids. Any source of nucleic acids, in purified or non-purified form, can be utilized as the starting nucleic acid, provided it contains or is suspected of containing the specific nucleic

acid sequence desired. DNA or RNA may be extracted from cells, tissues, body fluids and the like.

Methods of genotyping find use in, e.g., in genotyping case-control populations in association studies as well as in genotyping individuals in the context of detection of alleles of biallelic markers which are known to be associated with a given trait. In the context of the present invention, a preferred trait is a mental disorder selected from the group of bipolar disorder, schizophrenia and depression, and most preferably bipolar disorder.

Accordingly, a preferred embodiment is directed to a method of diagnosing a mental disorder in an individual comprising the step of genotyping at least one KCNQ2-related biallelic marker using a method of genotyping comprising the step of determining the identity of a nucleotide at a KCNQ2-related biallelic marker or the complement thereof in a biological sample derived from said individual. Such a diagnosing method may further comprise the step of correlating the result of the genotyping step with a risk of suffering from said mental disorder. Typically, the presence of the risk allele, risk genotype or risk haplotype of the genotyped KCNQ2-related biallelic marker(s) is correlated with a risk of suffering from the mental disorder. Preferably, said KCNQ2-related biallelic marker is selected from the group consisting of 30-2/62 and 30-7/30 and the complements thereof. In one embodiment, the presence of a genotype "AG" at biallelic marker 30-2/62218 is indicative of a risk of suffering from said mental disorder. In another embodiment, the presence of a genotype "CC" at biallelic marker 30-7/30 is indicative of a risk of suffering from said mental disorder. Preferably, the mental disorder is selected from the group consisting of bipolar disorder, schizophrenia and depression. Most preferably, the mental disorder is bipolar disorder.

The present invention is further directed to the use of at least one KCNQ2-2-related biallelic marker for determining the haplotype of an individual. When determining the haplotype of an individual, each single chromosome should be studied independently. Methods of determining the haplotype of an individual are well known in the art and include, e.g., asymmetric PCR amplification (Newton et al. (1989) *Nucleic Acids Res.* 17:2503-2516; Wu et al. (1989) *Proc. Natl. Acad. Sci. USA.* 86:2757-2760), isolation of single chromosome by limit dilution followed by PCR amplification (Ruano et al. (1990) *Proc. Natl. Acad. Sci. USA.* 87:6296-6300) and, for sufficiently close biallelic markers, double PCR amplification of specific alleles (Sarkar and Sommer, (1991) *Biotechniques.* 10:436-440).

Thus the present invention is further directed to the use of at least one KCNQ2-related biallelic marker for determining the haplotype of an individual. For example, a method for determining a haplotype for a set of biallelic markers in an individual may comprise the steps of: a) genotyping said individual for at least one KCNQ2 related biallelic marker, b) genotyping said individual for a second biallelic marker by determining the identity of the nucleotides at said second biallelic marker. Preferably, both markers are KCNQ2-related

## EXAMPLES

### EXAMPLE 1: Yeast two-hybrid screening

#### 1. Construction of pGBKT7-PPP2R2C

The full-length coding region of the *PPP2R2C* gene, which encodes the PP2A/B $\gamma$  subunit, was first amplified from a Human foetal brain cDNA library (Marathon-Ready cDNA, Clontech) with the two gene-specific primers of SEQ ID NO: 8 and of SEQ ID NO: 9. This first PCR product was then amplified with a new combination of primers of SEQ ID NO: 10 and of SEQ ID NO: 11. The amplified fragment encompassed nucleotides 52-1540 of the full-length cDNA, genbank accession number AF086924 extended, respectively, with *EcoRI* and *BamHI* cloning sites. The resulting 1503-bp fragment was digested with *EcoRI* and *BamHI*, purified and inserted into *EcoRI* and *BamHI* cloning sites of the pGBKT7 vector (Clontech).

#### 2. The Yeast Two-Hybrid Screening

A yeast two-hybrid screening was performed to find polypeptides interacting with the PP2A/B $\gamma$  subunit. The *Saccharomyces cerevisiae* strain AH109 (*MATa*, *trp1-901*, *leu2-3, 112*, *ura3-52*, *his3-200*, *gal4 $\Delta$* , *gal80 $\Delta$* , *LYS2 :: GAL1<sub>UAS</sub>-GAL1<sub>TATA</sub>-HIS3*, *GAL2<sub>UAS</sub>-GAL2<sub>TATA</sub>-ADE2*, *URA3 :: MEL1<sub>UAS</sub>-MEL1<sub>TATA</sub>-lacZ*) was transformed with the pGBKT7-PPP2R2C construction. A lithium acetate transformation procedure was done according to the manufacturer's instructions (Matchmaker Two-Hybrid system, Clontech). The *MATa* transformed cells expressing the bait were then mixed with a pretransformed Matchmaker Human brain cDNA library in the Y187 strain (*MAT $\alpha$* , *ura3-52*, *his3-200*, *ade2-101*, *trp1-901*, *leu2-3, 112*, *gal4 $\Delta$* , *met<sup>-</sup>*, *gal80 $\Delta$* , *URA3 :: GAL1<sub>UAS</sub>-GAL1<sub>TATA</sub>-lacZ*). Three independent mating were performed with respectively  $5 \cdot 10^6$ ,  $5 \cdot 10^6$  and  $2 \cdot 10^5$  clones of the Human brain cDNA library. The resulting diploid cells able to grow on SD/-Leu/-Trp medium containing plates were further selected onto the medium-stringency SD/-Leu/-Trp/-His selective medium for the identification of bait-prey interactions. Positive colonies were then picked up and plated onto the high-stringency SD/-Leu/-Trp/-His/-Ade selective medium. Only cDNA of colonies able to grow at the same time on SD/-Leu/-Trp and SD/-Leu/-Trp/-His/-Ade media was retained for sequencing and further studies.

#### 3. Results of the Yeast Two-Hybrid Screening

494 clones were obtained, sequenced and analyzed. Among these clones, the 2E11 and 1D3 clones comprised partial cDNAs encoding a novel splice variant of the KCNQ2 potassium channel. 2E11 comprised a cDNA encoding amino acids 433 to 643 of SEQ ID NO: 2, and 1D3 comprised a cDNA encoding amino acids 454 to 643 of SEQ ID NO: 2. The full-length splice variants were cloned and sequenced as described in Example 2.

Exon 12 of KCNQ2 is lacking in SEQ ID NO: 4. Exons 9 and 12 of KCNQ2 are lacking in SEQ ID NO: 6.

The insert of the 2E11 clone, which corresponds to a partial cDNA, comprises exons 13, 14 and 15b.

Table 1

Exon No.	SEQ ID NO:1	Encodes AA of SEQ ID NO:2	SEQ ID NO:3	Encodes AA of SEQ ID NO:4	SEQ ID NO:5	Encodes AA of SEQ ID NO:6	Encodes AA of SEQ ID NO: 7
1	1-296	1-98	1-296	1-98	1-296	1-98	1-98
2	297-387	100-129	297-387	100-129	297-387	100-129	100-129
3	388-514	130-171	388-514	130-171	388-514	130-171	130-171
4	515-690	173-230	515-690	173-230	515-690	173-230	173-230
5	691-816	231-272	691-816	231-272	691-816	231-272	231-272
6	817-927	273-309	817-927	273-309	817-927	273-309	273-309
7	928-1023	310-341	928-1023	310-341	928-1023	310-341	310-341
8	1024-1118	342-372	1024-1118	342-372	1024-1118	342-372	342-372
9	1119-1148	374-382	1119-1148	374-382	/	/	374-382
10	1149-1217	384-405	1149-1217	384-405	1119-1187	374-395	384-405
11	1218-1247	407-415	1218-1247	407-415	1188-1217	397-405	407-415
12	1248-1301	417-433	/	/	/	/	417-433
13	1302-1525	435-508	1248-1471	417-490	1218-1441	407-480	435-508
14	1526-1631	510-543	1472-1577	492-525	1442-1547	482-515	510-543
15	/	/	/	/	/	/	545-587
15b	1632-1929	545-643	1578-1875	527-625	1548-1845	517-615	/
16	/	/	/	/	/	/	588-629
17	/	/	/	/	/	/	630-872

### EXAMPLE 3: Yeast mating test

#### 1. Construction of vectors

##### 1.1. EX13-17, which comprises exons 13, 14, 15, 16 and 17.

The pGADT7-EX13-17 plasmid was constructed as follows: a 1414-bp fragment was first PCR-amplified from a Human total brain cDNA library (Marathon-Ready cDNA, Clontech) with two gene-specific primers of SEQ ID NO: 15 and of SEQ ID NO: 16. This first PCR product was then amplified with a second set of gene-specific primers of SEQ ID NO: 17 and 5' of SEQ ID NO: 18. These primers are extended, respectively, with *EcoRI* and *BamHI* cloning sites. After digestion with *EcoRI* and *BamHI* restriction enzymes, the 1338-bp

purified fragment was ligated to the same cloning sites of pGADT7 (Clontech).

*1.2. EX13-15, which comprises exons 13, 14 and 15.*

The pGADT7-EX13-15 plasmid was obtained as follows: a 484-bp fragment was PCR-amplified with primers of SEQ ID NO: 19 and of SEQ ID NO: 20, which are respectively extended with *EcoRI* and *BamHI* cloning sites, from the first PCR product of the pGADT7-EX13-17 construction. The resulting fragment was then digested with *EcoRI* and *BamHI*, purified, and ligated to the same cloning sites of pGADT7 (Clontech).

*1.3. EX16-17, which comprises exons 16 and 17.*

The pGADT7-EX16,17 plasmid was obtained as follows: a 883-bp fragment was PCR-amplified with primers of SEQ ID NO: 21 and of SEQ ID NO: 22, which are respectively extended with *EcoRI* and *BamHI* cloning sites, from the first PCR product of the pGADT7-EX13-17 construction. The resulting fragment was then digested with *EcoRI* and *BamHI*, purified, and ligated to the same cloning sites of pGADT7 (Clontech).

*1.4. EXsp15b, which comprises the region unique to exon 15b.*

The pGADT7-EXsp15b plasmid was constructed as follows : a 400-bp fragment was PCR-amplified with a primer of SEQ ID NO: 23 extended with *EcoRI* cloning site, and with a primer of SEQ ID NO: 24 from the pACT2-2E11 plasmid (see example 1). The resulting fragment was then digested with *EcoRI* and *XhoI*, purified, and ligated to the same cloning sites of pGADT7 (Clontech).

*1.5. EXco15, which comprises the region common to exon 15 and exon 15b.*

The pGADT7-EXco15 domain plasmid was constructed as follows: a 146-bp fragment was PCR-amplified with primers of SEQ ID NO: 25 and of SEQ ID NO: 26, which are respectively extended with *EcoRI* and *BamHI* cloning sites, from the pACT2-2E11 plasmid. The resulting fragment was then digested with *EcoRI* and *BamHI*, purified, and ligated to the same cloning sites of pGADT7 (Clontech).

*1.6. EX13-14, which comprises exons 13 and 14.*

The pGADT7-EX13-14 plasmid was constructed as follows: a 300-bp fragment was PCR-amplified with primers of SEQ ID NO: 27 and of SEQ ID NO: 28, which are respectively extended with *EcoRI* and *BamHI* cloning sites, from the pACT2-2E11 plasmid. The resulting fragment was then digested with *EcoRI* and *BamHI*, purified, and ligated to the same cloning sites of pGADT7 (Clontech).

## 2. Protocol of the yeast mating test

Yeast mating tests were performed to map the interaction domains between the different partners. The chosen *Saccharomyces cerevisiae* mating partner strains (AH109 and Y184) were transformed separately with the plasmids to be tested in combination with the plasmid of interest. The lithium acetate transformation procedure was done according to the manufacturer's instructions (Matchmaker Two-Hybrid system, Clontech). Transformants were

selected on the appropriate SD dropout medium (Clontech). One fresh colony of each type to use was picked from the working stock plates and both placed in one 1.5 ml microcentrifuge tube containing 0.5 ml of YPD medium (Clontech). Cells were then incubated for 24 hr at 30°C with shaking at 200 rpm. 100  $\mu$ l of a 1:100 dilution of the mating culture were then spread on the appropriate SD medium: SD/-Leu/-Trp, and SD/-Leu/-Trp/-His/-Ade. After 7 to 15 days of growth on selective medium positive colonies were counted.

### 3. Results of the direct mating tests between KCNQ2 polypeptides and PP2A/B $\gamma$

Mating tests between each of the above constructions and the pGBKT7-PPP2R2C construction described in example 1 were performed. The results are shown on Figure 2. The sign "+" indicates that colonies grew, thus indicating that the tested polypeptide is capable of interacting with PP2A/B $\gamma$ . The sign "-" indicates that no colony grew, thus indicating that the tested polypeptide does not interact with PP2A/B $\gamma$ .

EX13-17, EX16-17, EX13-14 and EXsp15b do not interact with PP2A/B $\gamma$ . EX13-15b, EX13-15 and EXco15 interact with PP2A/B $\gamma$ . EX13-15b interacts with PP2A/B $\gamma$ , showing that KCNQ2-15b polypeptides are capable of interacting with PP2A/B $\gamma$ . Since EX13-15b, EX13-15 and EXco15 but *not* EXsp15b interact with PP2A/B $\gamma$ , the common region between exon 15 and exon 15b plays a role in this interaction. Furthermore, since EX13-17 does not interact with PP2A/B $\gamma$ , the fact that exon 15 or that exon 15b is located at the most carboxyl extremity of the KCNQ2 polypeptide is of importance for efficient interaction with PP2A/B $\gamma$ .

### 4. Results of the direct mating tests between different KCNQ2 polypeptides

Mating tests between the different above constructions were performed, and the results are shown on Figure 4. 4 mating tests were performed for each pair of constructs and the results are shown on Figure 3. The sign "++" indicates that all 4 colonies grew. The sign "+" indicates that 3 colonies out of 4 grew. The sign "-/+" indicates that 1 colony out of 4 grew. The sign "-" indicates that no colony grew.

This experiment shows that KCNQ2-15b polypeptides can associate and form homodimers. KCNQ2-15b polypeptides can also associate and form heterodimers with KCNQ2 polypeptides comprising exon 15 at their carboxyl-terminal extremity. KCNQ2-15b polypeptides associate with KCNQ2-fl polypeptides at a lesser extent.

## **EXAMPLE 4: Expression and Purification of Glutathione S-Transferase Fusion Proteins**

### 1. Construction of plasmids

#### 1.1. pGBKT7-2E11

The pACT2-2E11 plasmid rescued from yeast two-hybrid screening was digested with *EcoRI* and *BglII* and the resulting 687-bp fragment inserted after purification into *EcoRI* and

cDNA, genbank accession number AF033348) was first amplified from a Human brain cDNA library (Marathon-Ready cDNA, Clontech) using gene specific primers of SEQ ID NO: 35 and of SEQ ID NO: 36, which are respectively extended with *EcoRI* and *BglII* cloning sites. The PCR product was digested with *EcoRI* and *BglII*, purified and ligated to the same cloning sites of the pCMV-HA vector (Clontech). The protein encoded by pGEX-2TK-2E11 is named GST-KCNQ2-Cter.

## 2. Expression and purification

Glutathione S-transferase fusion protein expression and purification by adapting the method described by Kaelin et al. (1991, Cell, 64:521-532). Overnight cultures of MAX Efficiency DH5 $\alpha$ F'IQ competent cells (Invitrogen) transformed with either the pGEX2TK plasmid or the pGEX2TK-2E11, pGEX2TK-KCNQ2-Cter, and pGEX2TK-PPP2R2C recombinants were diluted 1:10 in LB medium containing ampicillin (100  $\mu$ g/ml) and incubated for 1 hr at 37°C. Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG, Promega) was then added to a final concentration of 0.1 mM and bacteria let to grow for 3 additional hours at 37°C. For fusion proteins recovery using the glutathione-Sepharose 4B beads (Amersham Biosciences), bacterial cultures were pelleted by centrifugation at 5000 x g for 15 min at 4°C and resuspended in 1/10 vol NETN (20mM Tris-HCl [pH 8.0], 120mM NaCl, 1mM EDTA, 0.5% Nonidet P-40) supplemented with 1mM phenylmethylsulfonyl fluoride (PMSF, Sigma) and one tablet of protease inhibitors cocktail (Complete™ mini, Roche) for 7 ml of buffer. The bacteria were then lysed on ice by mild sonication and centrifuged at 10,000 x g for 10 min at 4°C. Aliquots (1 ml) of bacterial clear lysates were then rocked for 1 hr at 4°C with 50  $\mu$ l of glutathione-Sepharose 4B beads, which had been previously washed four times in NETN containing 1% Albumin Bovine (BSA fraction V, Sigma) and resuspended (final concentration 1:1 [v/v]) in NETN. The glutathione-Sepharose 4B beads were then washed three times with NETN. For recovery of the bound recombinants proteins, beads were washed two more times with 100mM Tris-HCl [pH 8.0], 120 mM NaCl and elution was performed in the same buffer containing 20 mM glutathione (Sigma). Quantification of the eluted fusion proteins was performed by the standard Bradford's method (Biorad Protein Assay).

### **EXAMPLE 5: In vitro Labeling of the GST Fusion Proteins**

Beads with bound GST fusion proteins corresponding to 1 ml of bacterial clear lysate were washed three times in NETN and one time with HMK buffer without DTT (20 mM Tris-HCl [pH 7.5], 120 mM NaCl, 12 mM MgCl<sub>2</sub>). Beads were then resuspended in 30  $\mu$ l of reaction mix (3  $\mu$ l of 10X HMK Buffer with 20 mM DTT, 10 units of Protein Kinase A Catalytic Subunit [PKA from bovine heart, 250 units/vial, Sigma] in 40mM DTT, 2  $\mu$ l of [<sup>32</sup>P]- $\gamma$ ATP 6000 Ci/mMole and 24  $\mu$ l of distilled water) and incubated at 4°C for 30 min. During incubation

## 2. Immunoprecipitation

500 µg (final volume: 500 µl) of the clear cell lysate were incubated for 2 hr at 4°C with 1 µl of rabbit preimmune serum and 50 µl of protein A Sepharose CL-4B beads (Amersham Pharmacia Biotech) saturated with 1% Albumin Bovine (BSA fraction V, Sigma). Depleted supernatants were then incubated overnight at 4°C with 1 µg of anti-Myc monoclonal antibody (Myc-Tag 9B11 monoclonal antibody, Cell Signaling). Protein A Sepharose CL-4B beads saturated with 1% Albumin Bovine were then added and the mixture incubated at 4°C for 2 additional hours. After five washes with ice-cold solubilization buffer immuno-complexes were boiled in 2X Sample Buffer (125 mM Tris-HCl [pH 6.8], 4% SDS, 20% glycerol, 1.4 M β-Mercapto ethanol), resolved by 8% SDS-PAGE and subjected to

## 3. Western blot

Proteins were transferred onto nitrocellulose membrane (nitrocellulose transfer membrane Protran BA 83, Schleicher and Schuell) using Towbin buffer (Towbin et al., 1979, PNAS, 76:4350-4354) and an electrotransfer device. After transfer, membranes were blocked, in 5% non-fat dried milk in TBST (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.05% Tween 20) supplemented with sodium azide (0.1%) for 2 hr, and then incubated for 16 hr at room temperature with the anti-Myc monoclonal antibody (Myc-Tag 9B11 monoclonal antibody, Cell Signaling) diluted 1:1000 in the same buffer. After several washes with TBST, the blot was incubated with a horseradish peroxidase-conjugated secondary antibody (Anti-mouse IgG, Fab specific, peroxidase conjugate, Sigma) diluted 1:5000 and developed using ECL Western blotting detection reagents (Amersham Biosciences).

## **EXAMPLE 9: Electrophysiological Analysis**

### 1. Protocols

#### 1.1. *cDNA injection in Xenopus laevis oocytes*

The animal was anesthetized and pieces of the ovary were surgically removed and individual oocytes were dissected away in a saline solution (ND96) containing 96 mM NaCl, 2 mM KCl, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub> and 5 mM HEPES at pH 7.4. Stage V and VI oocytes were treated at room temperature for 2h with collagenase type 1A (1mg/ml) in the presence of 0.2 mg/ml trypsin inhibitor in saline solution to discard follicular cells. The concentrations were determined by measuring the absorbance at 260 nm. DNA corresponding to KCNQ2, 3H2 and 3H9 K<sup>+</sup> channels were subcloned in PEKO vector in order to generate the respective cRNAs. cRNA concentrations were measured by absorbance at 260nm. cRNA solutions were injected (about 50 nL/oocyte) using a pressure microinjector (Inject+matic, Genève). Oocytes were then kept for 2-6 days in ND96 solution supplemented with 50U/mL penicillin and 50 U/mL streptomycin.

**Table 4A: Primers in the PPP2R2C gene**

amplified region	Marker name	Orientation of the primer	Position of the primer on SEQ ID NO: 37	SEQ ID No. of the primer
24-257	24-257/320	(+1)	109644 to 109662	SEQ ID NO: 40
99-24169	99-24169/139	(+1)	84007 to 84025	SEQ ID NO: 39
99-24175	99-24175/218	(+1)	117441 to 117459	SEQ ID NO: 41
24-247	24-247/216	(+1)	99486 to 99504	

**Table 4B: Primers in the KCNQ2 gene**

amplified region	Marker name	Orientation of the primer	SEQ ID No.	Position of the primer on indicated SEQ ID No.
30-4	30-4/58	(-1)	SEQ ID NO: 42	302 to 319 (primer B18)
30-4	30-4/58	(+1)	SEQ ID NO: 42	282 to 300 (primer A19)
30-2	30-2/62	(-1)	SEQ ID NO: 43	302 to 320
30-17	30-17/37	(-1)	SEQ ID NO: 44	302 to 324
30-7	30-7/30	(+1)	SEQ ID NO: 45	280 to 300
30-84	30-84/37	(-1)	SEQ ID NO: 46	302 to 318
30-15	30-15/54	(-1)	SEQ ID NO: 47	302 to 323

As for the primers in tables 2A and 2B, the sign (+1) in the column "orientation" indicates that the sequence of the primer is identical to the corresponding region of SEQ ID Nos. 37 and 42 to 47, and the sign (-1) indicates that the sequence of the primer is complementary to the corresponding region of SEQ ID Nos. 37 and 42 to 47.

The microsequencing reaction performed as follows. After purification of the amplification products, the microsequencing reaction mixture was prepared by adding, in a 20 µl final volume: 10 pmol microsequencing oligonucleotide, 1 U Thermosequenase (Amersham E79000G), 1.25 µl Thermosequenase buffer (260 mM Tris HCl pH 9.5, 65 mM MgCl<sub>2</sub>), and the two appropriate fluorescent ddNTPs (Perkin Elmer, Dye Terminator Set 401095) complementary to the nucleotides at the polymorphic site of each biallelic marker tested, following the manufacturer's recommendations. After 4 minutes at 94°C, 20 PCR cycles of 15 sec at 55°C, 5 sec at 72°C, and 10 sec at 94°C were carried out in a Tetrad PTC -225 thermocycler (MJ Research). The unincorporated dye terminators were then removed by ethanol precipitation. Samples were finally resuspended in formamide-EDTA loading buffer and heated for 2 min at 95°C before being loaded on a polyacrylamide sequencing gel. The data were collected by an ABI PRISM 377 DNA sequencer and processed using the GENESCAN software (Perkin Elmer).

Following gel analysis, data were automatically processed with software that allows the determination of the alleles of biallelic markers present in each amplified fragment.

The software evaluates such factors as whether the intensities of the signals resulting from the above microsequencing procedures are weak, normal, or saturated, or whether the signals are ambiguous. In addition, the software identifies significant peaks (according to shape and height criteria). Among the significant peaks, peaks corresponding to the targeted site are identified based on their position. When two significant peaks are detected for the same position, each sample is categorized classification as homozygous or heterozygous type based on the height ratio.

### **EXAMPLE 15: Association Study Between Bipolar Disorder And The Biallelic Markers Of The Invention**

#### **5.1. Collection of DNA Samples From Affected And Non -Affected Individuals**

The association studies were performed on two different populations. One collection of samples was provided by Hospital Pinero, Buenos-Aires, Argentina (the "Labimo" collection). The other collection of samples was provided by the University College of London (the "UCL" collection). Both collections are constituted by individuals that are affected or not by bipolar disorder.

##### A) The Labimo collection

###### a) Affected population

206 DNA samples from patients suffering from bipolar disorder (cases) were collected for genotyping analysis.

All patients fulfilled DSM-IV and ICD-10 criteria for bipolar type I (ICD-10: F30.x, F31.x) or bipolar type II (ICD-10: F31.8). All patients were of Caucasian ethnic origin up to the 2<sup>nd</sup> generation.

All potential patients suffering from a medical disorder or from a drug abuse were excluded.

According to DSM-IV criteria, 115 cases were classified as bipolar type I, 69 were bipolar type II, 22 were unclassified, and information concerning the type of bipolar disorder was lacking in 20 cases (8.5%)

The main phenotypic data of the cases were as follows:

- Mean age at first symptoms: 25.6 years (SD, 11; range, 8 -58)
- Mean age at inclusion: 43.3 years (SD, 13.8; range, 17 -76)
- Gender: 142 females and 84 males (ratio, 1.7)
- Ethnic origin: 213 were European Caucasian, 7 were non-European Caucasians, and information was lacking in 6 cases (2.5%)

collection (significant genotypic p-value). 24-247/216 is associated with bipolar disorder in the UCL collection (significant genotypic p-value).

The risk allele for the 99-24169/139 biallelic marker is "A". The risk alleles for the 24-257/320 biallelic marker and for the 99-24175/218 biallelic marker are also "A". The risk genotype for the 99-24169/139 biallelic marker is "AA". Thus an individual carrying the genotype "AA" at biallelic marker 99-24169/13 is at risk of developing bipolar disorder.

#### Biallelic markers in the KCNQ2 gene

Two biallelic markers located in the KCNQ2 gene, 30-2/62 and 30-7/30, are associated with bipolar disorder. More specifically, 30-2/62 is found to be highly associated with bipolar disorder in the UCL collection (significant allelic and genotypic p-values). 30-7/30 is associated with bipolar disorder in the UCL collection (significant genotypic p-value).

The risk genotype for 30-2/62 is "AG". The risk genotype for 30-7/30 is "CC". Thus individuals carrying the genotype "AG" at biallelic marker 30-2/62 and individuals carrying the genotype "CC" at biallelic marker 30-7/30 are at risk of developing bipolar disorder.

The association results of the single biallelic marker frequency analysis show that both the PPP2R2C gene and the KCNQ2 gene are associated with bipolar disorder. Accordingly, deregulation and/or dysfunction of KCNQ2 polypeptides and PP2A phosphatases comprising the PP2A/B $\gamma$  regulatory subunit contribute to the onset and to the development of bipolar disorder.

#### **C) Haplotype frequency analysis**

The analysis of haplotype frequencies cannot readily be derived from observed genotypic data. The EM (Expectation-Maximization) algorithm (Excoffier L & Slatkin M, 1995) allows the estimation of haplotypes for the population under investigation. Haplotype frequency estimations were performed by applying the OMNIBUS likelihood ratio test (PCT publication WO 01/091026)

The haplotype analysis was performed for two sets of markers located in PPP2R2C. The haplotype analysis for 24-257/320 and 99-24175/218 was performed in the Labimo collection. The haplotype analysis for 99-24169/139 and 24-247/216 was performed in the UCL collection. The results are shown in tables 7 (p-values) and 7B (odds ratios).

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,442,519

Page 1 of 3

APPLICATION NO.: 10/519,335

DATED : October 28, 2008

INVENTORS : Laurent Cavarec, Ilya Chumakov, Benoit Destenaves, Catherine Gonthier, Isabelle Elias

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 12,

Line 62, “ “Phenotype” ” should read --“phenotype”--.

Column 14,

Line 2, “such assay s” should read --such assays--.

Line 12, “alter native” should read --alternative--.

Column 16,

Line 2, “ncbi.nim.nih.gov)” should read --ncbi.nlm.nih.gov)--.

Column 17,

Line 47, “substancially the same” should read --substantially the same--.

Column 18,

Line 37, “complementary thereto” should read --complementary thereto--.

Line 43, “complementary thereto” should read --complementary thereto--.

Lines 49-50, “to a polynucleotides” should read --to a polynucleotide--.

Line 63, “NO; 3” should read --NO: 3--.

Column 23,

Line 23, “Accession No. 043526” should read --Accession No. O43526--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik

P.O. Box 142950

Gainesville, FL 32614-2950

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,442,519

Page 2 of 3

APPLICATION NO.: 10/519,335

DATED : October 28, 2008

INVENTORS : Laurent Cavarec, Ilya Chumakov, Benoit Destenaves, Catherine  
Gonthier, Isabelle Elias

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 25,

Lines 11-12, "ho momeric" should read --homomeric--.

Line 53, "that (I)" should read --that (i)--.

Column 28,

Line 56, "consisting of 30-2162" should read --consisting of 30-2/62--.

Column 30,

Line 1, "marker 30-7130" should read --marker 30-7/30--.

Line 67, "Human fcetal" should read --Human foetal--.

Column 31,

Line 33, "SDI-Leu/-Trp and SDI-Leu/-Trp/-His/-Ade" should read  
--SD/-Leu/-Trp and SD/-Leu/-Trp/-His/-Ade--.

Line 35, "the He Yeast" should read --the Yeast--.

Column 33,

Line 32, "digestion With EcoRi" should read --digestion with EcoRi--.

Line 61, "pGAD7" should read --pGADT7--.

Column 34,

Line 42, "10 µl" should read --100 µl--.

Line 44, "SDI-Leu/-Trp/-His/-Ade" should read --SD/-Leu/-Trp/-His/-Ade--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik

P.O. Box 142950

Gainesville, FL 32614-2950

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,442,519

Page 3 of 3

APPLICATION NO.: 10/519,335

DATED : October 28, 2008

INVENTORS : Laurent Cavarec, Ilya Chumakov, Benoit Destenaves, Catherine  
Gonthier, Isabelle Elias

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 36,

Line 50, "w performed" should read --was performed--.

Column 37,

Lines 19-20, "membrane were then blocked" should read  
--membrane was then blocked--.

Line 66, "phsophorylation" should read --phosphorylation--.

Column 39,

Line 9, "phophorylation" should read --phosphorylation--.

Column 40,

Line 17, "1 µd" should read --1 µl--.

Column 45,

Line 57, "10 µmol" should read --10 pmol--.

Column 46,

Line 20, "dassification" should read --classification--.

Column 50,

Line 64, "for 30-7130" should read --for 30-7/30--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik

P.O. Box 142950

Gainesville, FL 32614-2950